IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Parent Application of:
Yoshiaki ISOBE et al.:
Application No. 10/528,343: Art Unit: 1624
Filed: March 18, 2005: Examiner: BERCH, Merk L
For: NOVEL ADENINE COMPOUND AND USE THEREOF

DECLARATION

Honorable Commissioner for Patents

Sir:

I, Yoshiaki ISOBE, a citizen of Japan residing in Takatsuki-shi, Osaka-fu, Japan, declare as follows:

I graduated from The Gifu Pharmaceutical University in 1985, and have obtained the doctor in said university in 2003.

Since 2002 up till now, I have been employed in Dainippon Sumitomo Pharma Co., Ltd. and I have engaged in research and development of Immunology.

I am an author of the publications as shown below.

"Synthesis and biological evaluation of novel 9-substituted-8-hydroxyadenine derivatives as potent interferon inducers." J. Med. Chem., 2006; 49: 2088-2095.

"Structure and activity relationships of novel uracil derivatives as topical anti-inflammatory agents." Bioorg. Med. Chem., 2003; 11: 4933-4940.

"Synthesis and structure-activity relationships of 2-substituted-8-hydroxyadenine derivatives as orally available interferon inducers without emetic side effects." Bioorg. Med. Chem., 2003; 11: 3641-3647.

"Inhibitory activities of novel pyrimidine derivatives on the contact hypersensitivity reaction." Chem. Pharm. Bull., 2003; 51: 309-312.

"Discovery of 8-hydroxyadenines as a novel type of interferon inducer." J. Med. Chem., 2002; 45: 5419-5422.

I am one of inventors of the present invention (U.S. Application No. 10/528,343) and familiar with the subject matter thereof.

Under my supervision, the following tests were carried out.

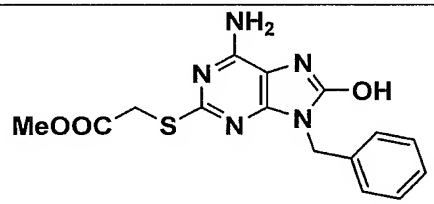
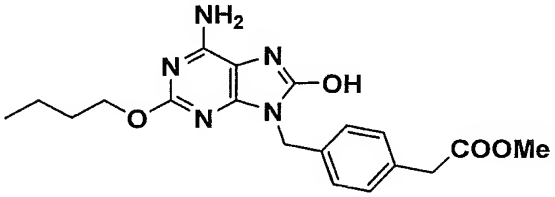
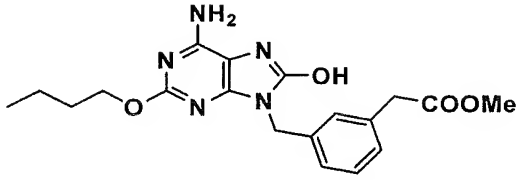
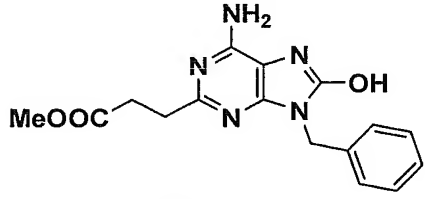
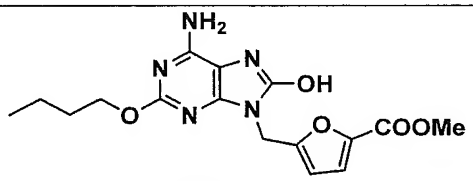
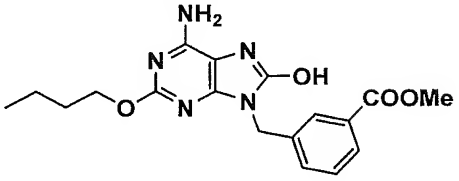
1. IFN inducing activity in vitro

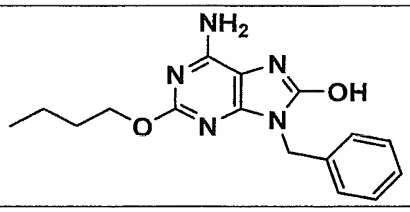
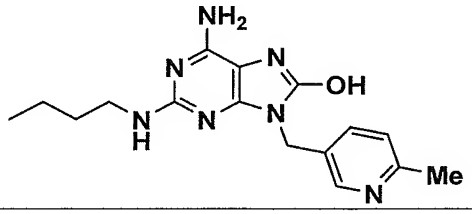
Test compounds are shown in Table 1.

Compounds A to F are included in the formula (1) of the present invention and were prepared in accordance with the method of the example indicated respectively.

Comparative compounds 1 and 2 have not an ester group and are not included in the formula (1) of the present invention and were prepared in a similar manner as examples of the present invention.

Table 1

	Structure	Minimum effective concentration of IFN inducing activity
Compound A (Example 40)		10nM
Compound B (Example 20)		0.1nM
Compound C (Example 19)		0.3nM
Compound D (Example 61)		3nM
Compound E (Example 15)		1nM
Compound F (Example 1)		0.3nM

Comparative compound 1		1nM
Comparative compound 2		3nM

Test method

The method was carried out in accordance with the method disclosed in Example 122 of the present invention.

Result

Comparative compounds 1 and 2 show the same interferon inducing activity as the compounds represented by the formula (1) of the present invention.

2. Pharmacological activity in vivo and plasma concentration of IFN

Pharmacological evaluation on eosinophile infiltrated model BN rats

Test compound

The test was carried out on compounds A, B and C and comparative compound 2.

Test method

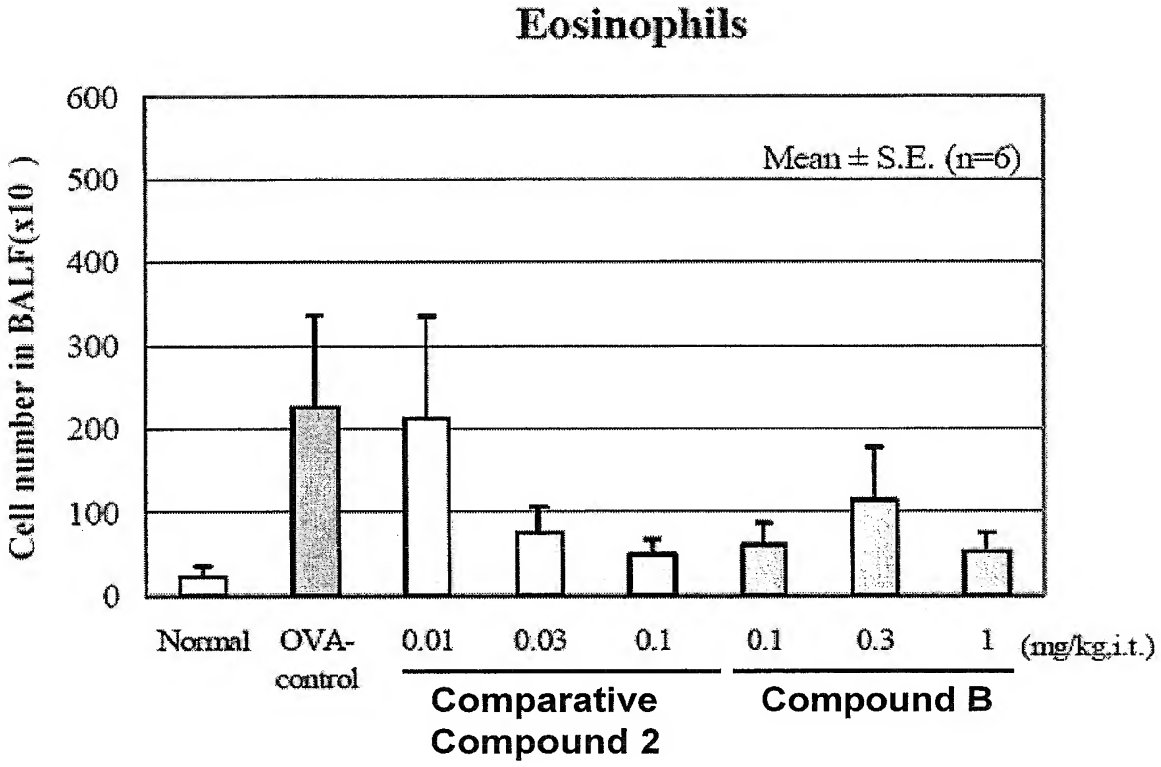
One ml of a solution of ovalbumin (1mg) and aluminum hydroxide (100mg) (Wako Chemical) in saline solution was intraperitoneally administered to BN/Crj rat at 1st day and 7th day. Saline solution was intraperitoneally administered to an unchallenged group.

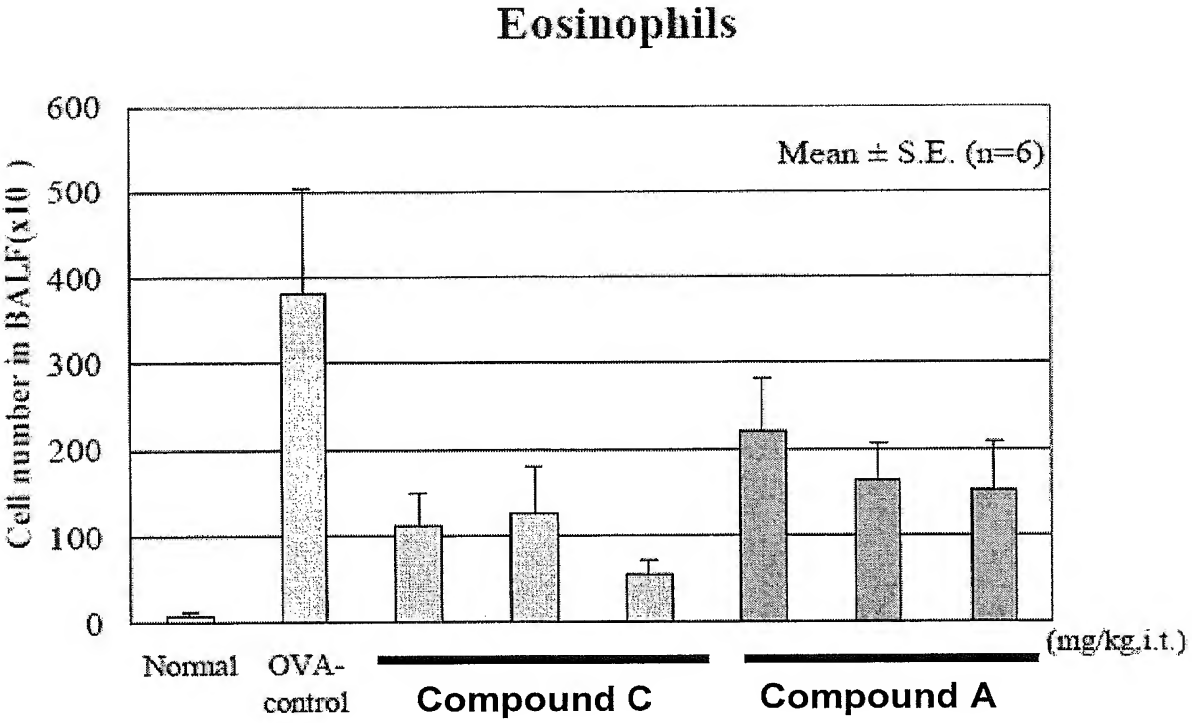
A test compound suspended in saline solution was bronchially administered to the test group, and saline solution was bronchially administered to the control group in the amount of 0.5ml/kg at 2.5 hours before the secondary administration of OVA. On 14th day, 1% OVA solution was rechallenged by inhalation with an ultrasonic nebulizer. Saline solution was administered to the group without challenge.

Twenty four hours later after antigen-challenge bronchoalveorar lavege fluid (BALF) was collected and rate (%) of eosinophil was calculated by sitespine sample.

Result

The inhibitory activity of eosinophil infiltration on BN rats was shown in the following table. As shown in the table, all of compounds A, B, C and comparative compound 2 showed the inhibitory activity of eosinophil infiltration.





3. IFN induction into plasma after the compound of the present invention was bronchially administered to BN rats

Test compound

The test was carried out on compounds shown in Table 1.

Test method

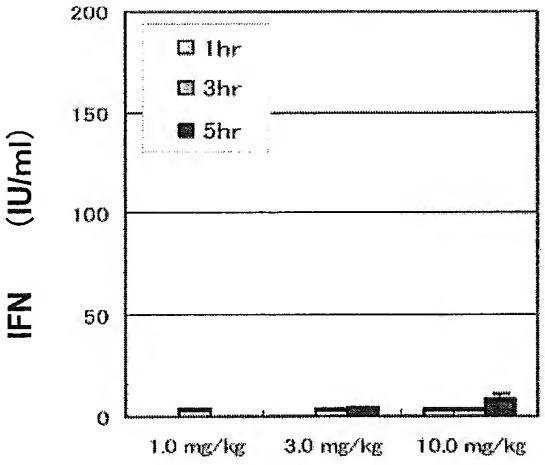
At 3, 5 and 7 hours after test compounds: compounds A, B, C, D, E and F, and comparative compound 2 were administered, blood was collected and the amount of IFN was measured in accordance with the method described in Example 122 of the present invention.

Result

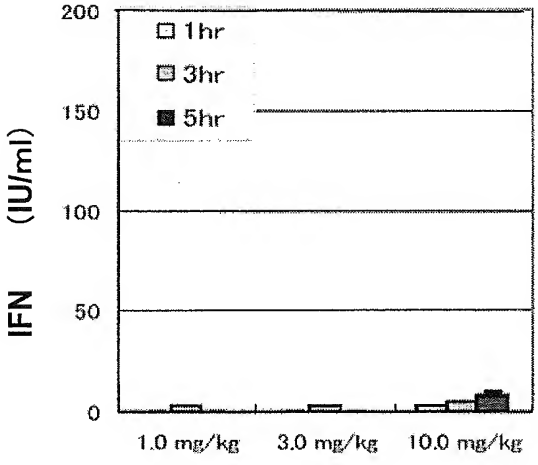
The plasma concentration of IFN in BN rats were shown below.

When the compounds of the formula (1) of the present invention (3mg/kg or less than 3mg/kg) were bronchially administered, IFN in plasma was hardly detected. Namely, when the compound of the formula (1) of the present invention is bronchially administered, the compound shows pharmacological activity, but plasma concentration of IFN is not raised.

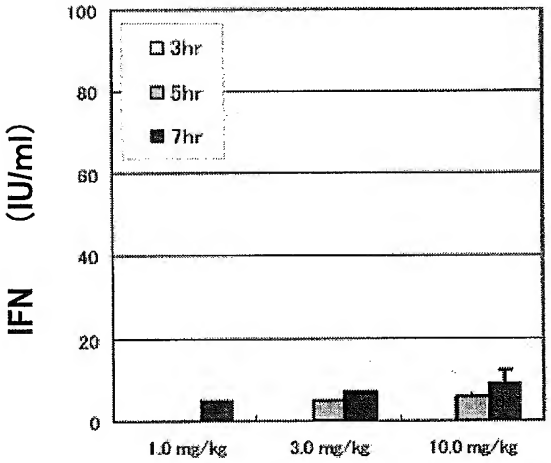
Compound A



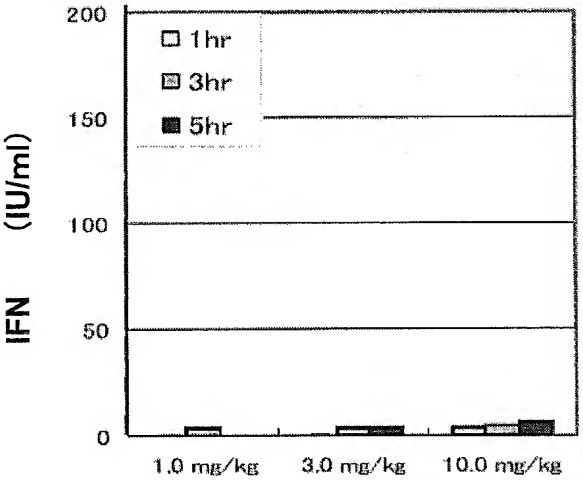
Compound B



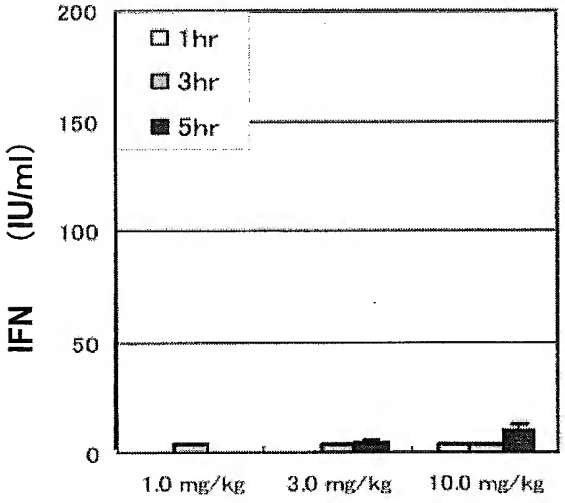
Compound C



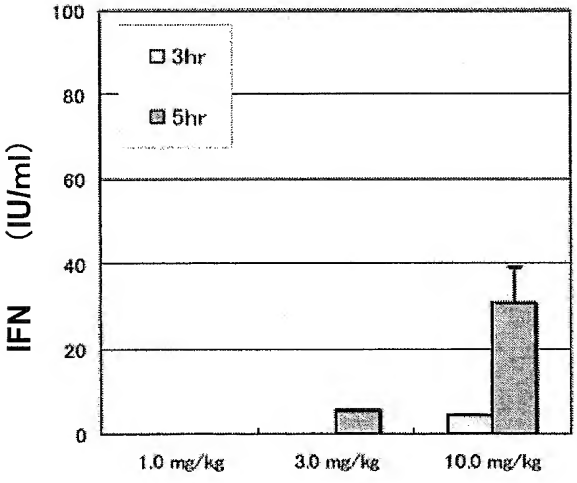
Compound D



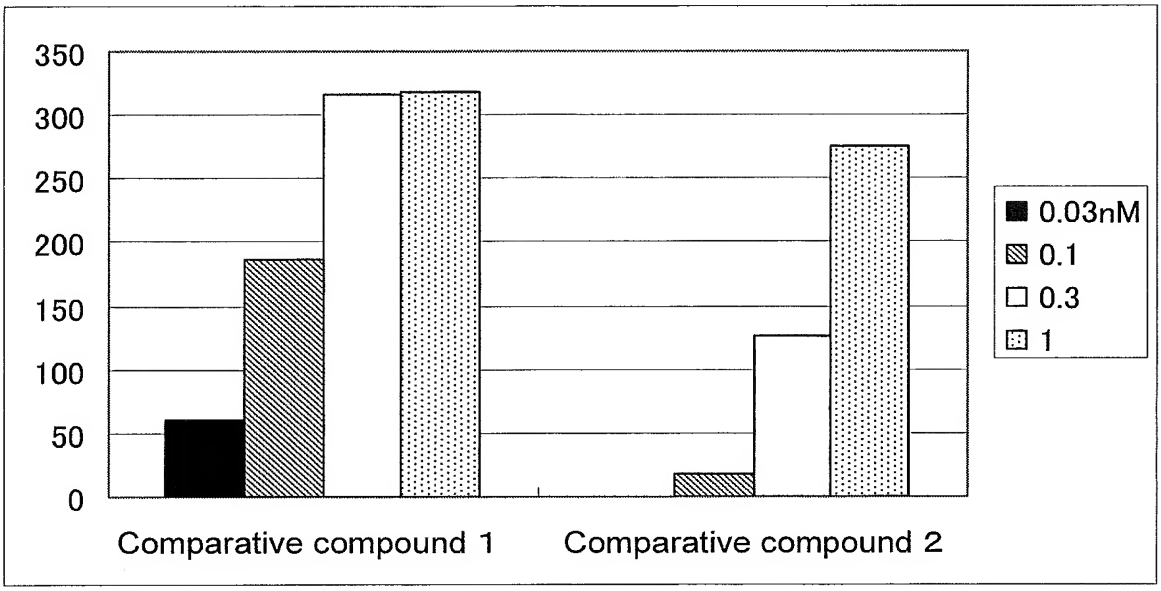
Compound E



Compound F

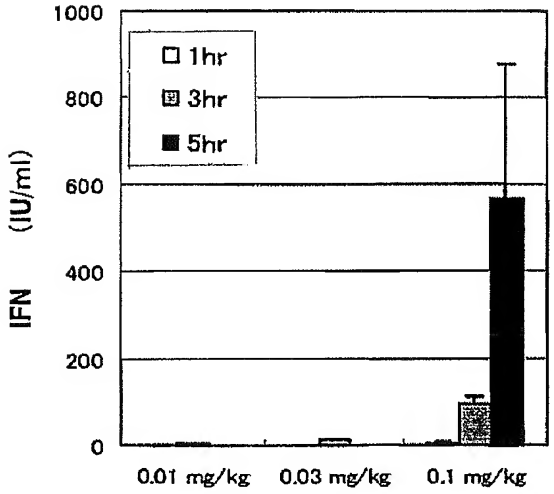


On the other hand, when comparative compounds 1 and 2 were orally administered, IFN was induced and plasma concentration of IFN was raised. For example, even when the comparative compound was administered in the amount of 0.1mg/kg, which corresponds to about 1/100 of the compound of the present invention, plasma concentration of IFN was raised.



Even when the comparative compound is bronchially administered, the same phenomenon is observed. Namely, the comparative compound was bronchially administered and its plasma concentration of IFN was shown below. Said compound induces IFN at low concentration such as 0.1mg/kg as shown below.

Comparative compound 2



The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1000 of Title 18 of the United State Code and that such willful false statements may jeopardize the validity of the above-mentioned application or any patenting thereon.

This 8 day of July, 2008

Yoshiaki Isobe

Dr. Yoshiaki ISOBE